NTP
National Toxicology Program

Study Designs for Reproductive and Developmental Toxicity: The Modified One-Generation Study

Paul Foster, PhD

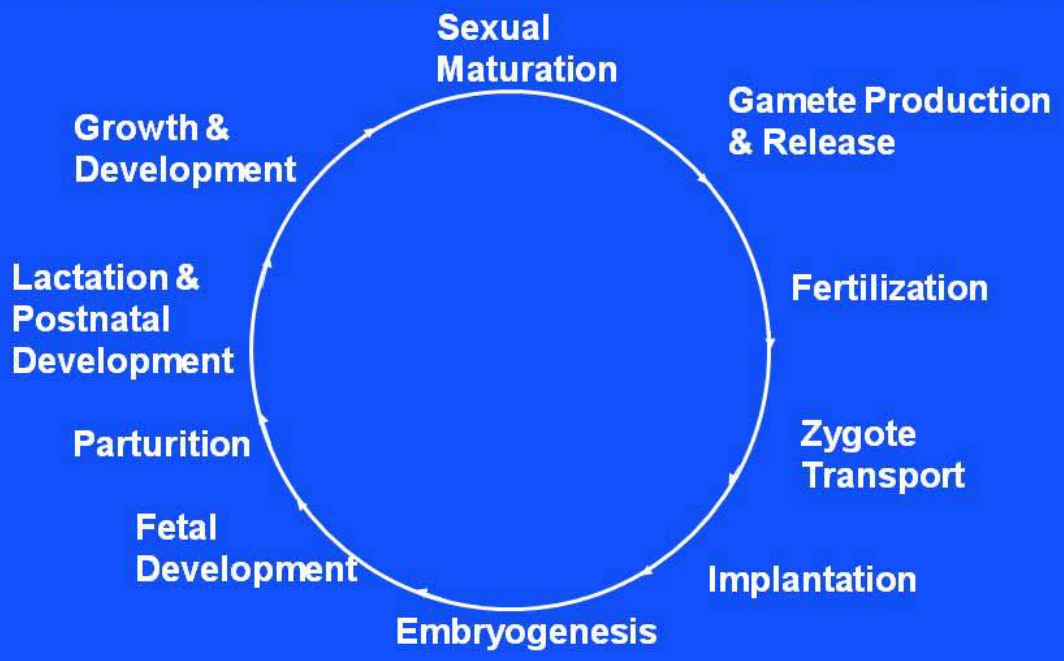
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors, April 13, 2011

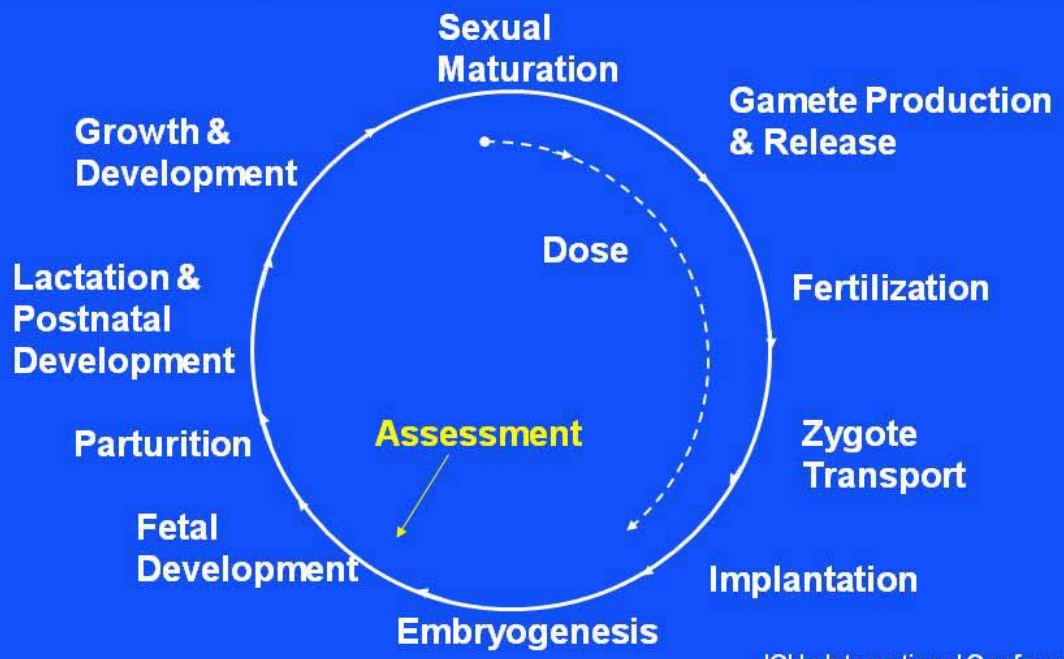
Overview

- Brief review of the principles behind the study designs for reproductive and developmental toxicity
- Recent changes in NTP study designs for toxicity and carcinogenicity in rats
  - Using perinatal exposure as a default
- Outline of the NTP modified one-generation (MOG) study design
  - How it better utilizes animals already produced and is consistent with the principles of the 3R's
- Comparison with the proposed OECD extended one-generation study currently in review

THE REPRODUCTIVE CYCLE



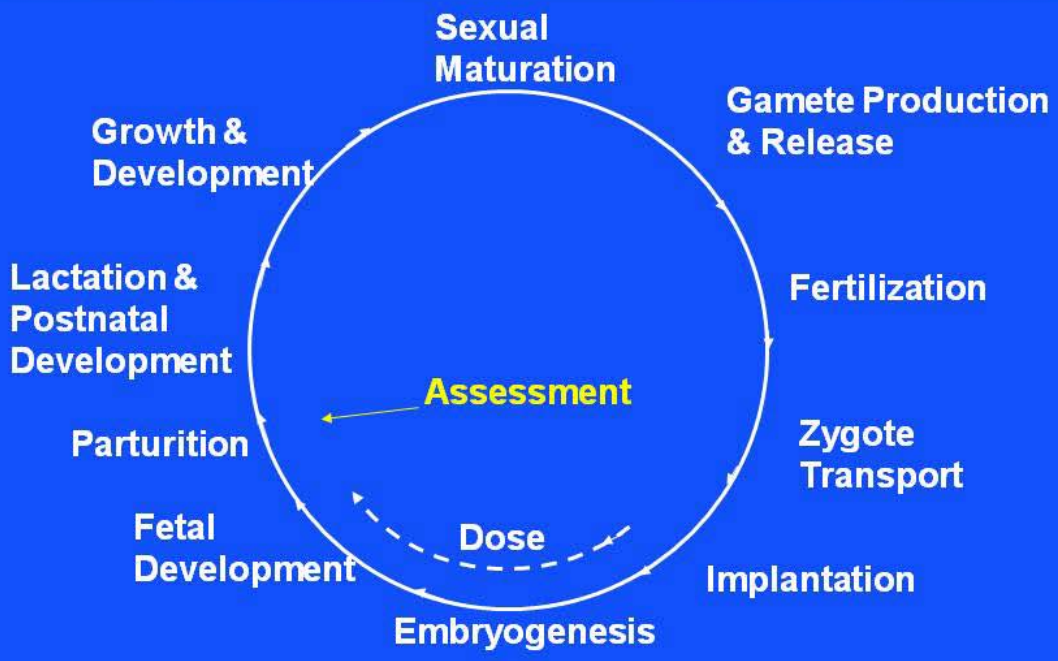
ICH Fertility & Early Embryonic Development Study (Seg I)



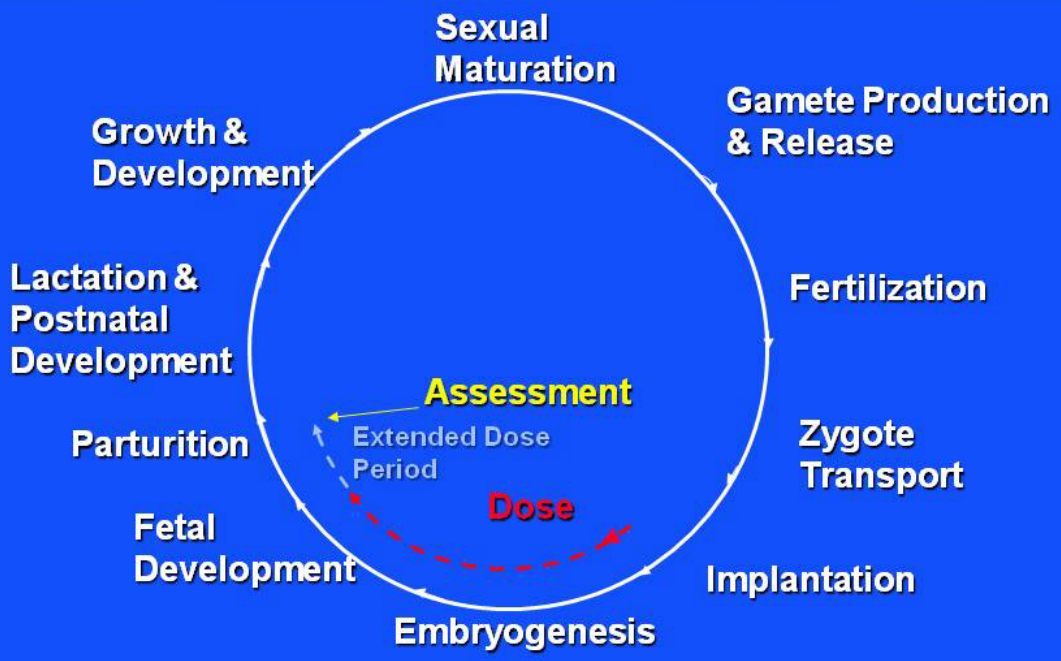
PMD Foster

ICH= International Conference on Harmonization of Guidelines

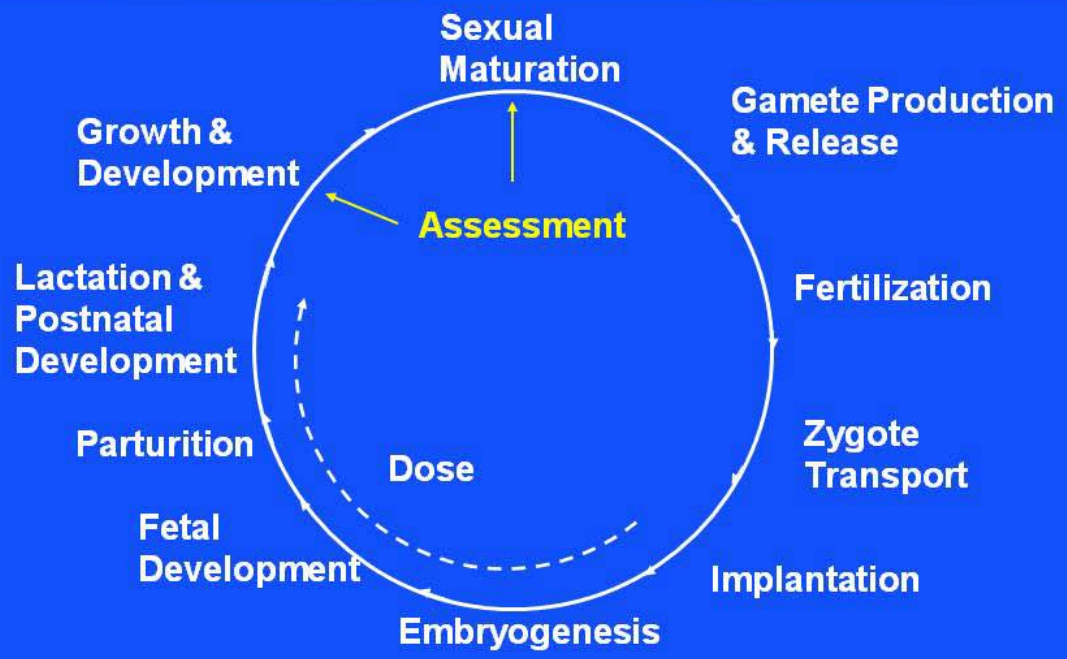
ICH Embryo-Fetal Development Study (Seg II)



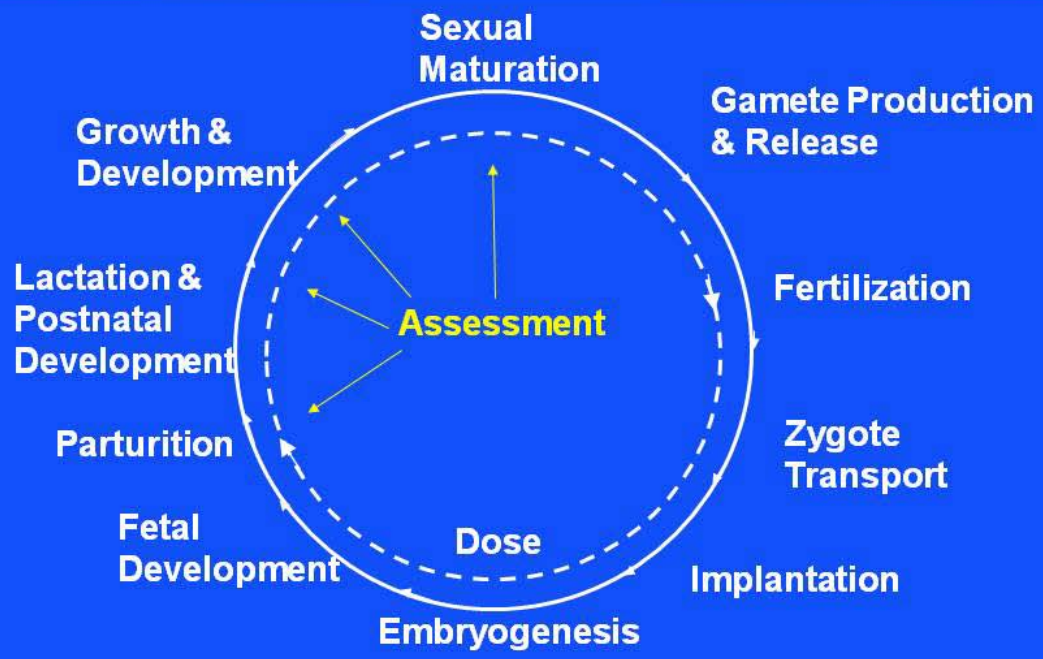
EPA / OECD Prenatal Developmental Toxicity Study



ICH Pre- and Post-natal Development Study (Seg III)

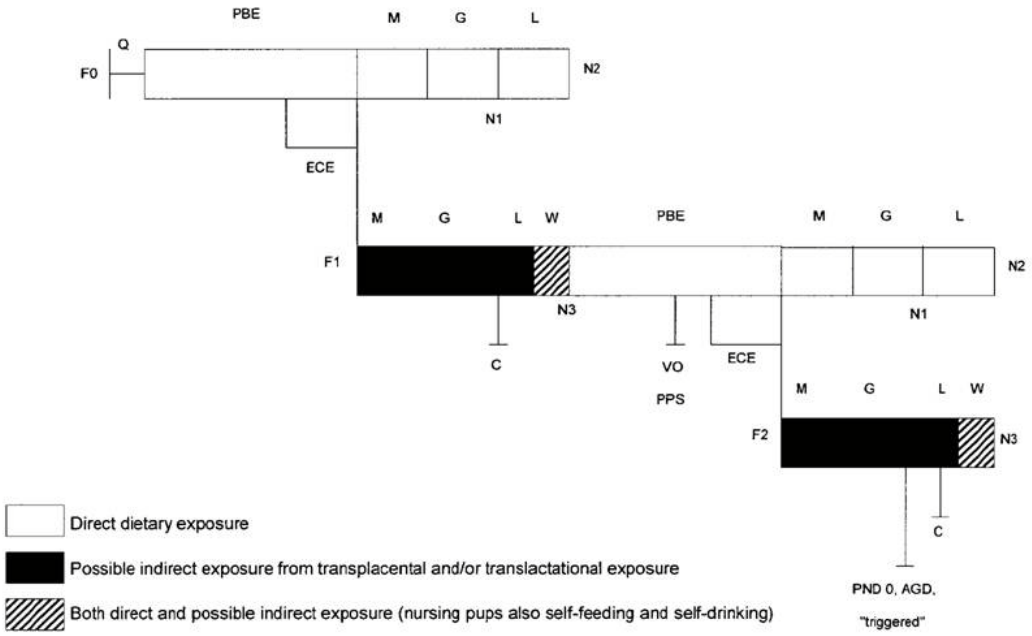


EPA / OECD Multigeneration Reproduction Study



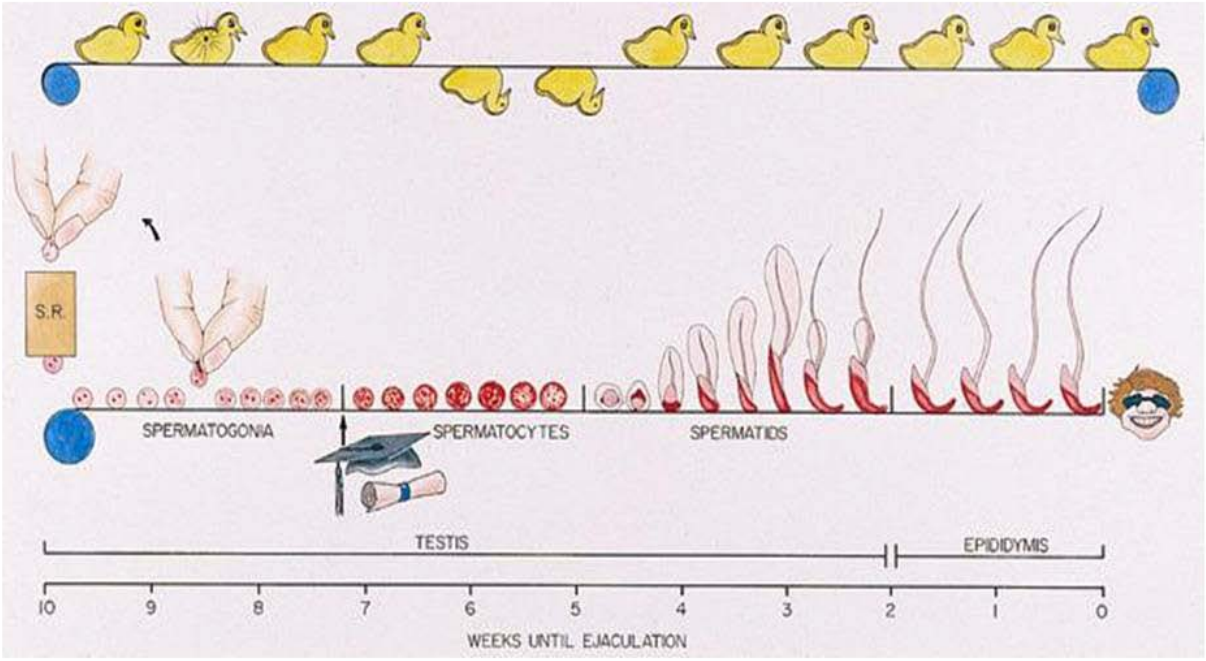
NTP
National Toxicology Program

EPA / OECD Multigeneration Reproduction Study



NTP
National Toxicology Program

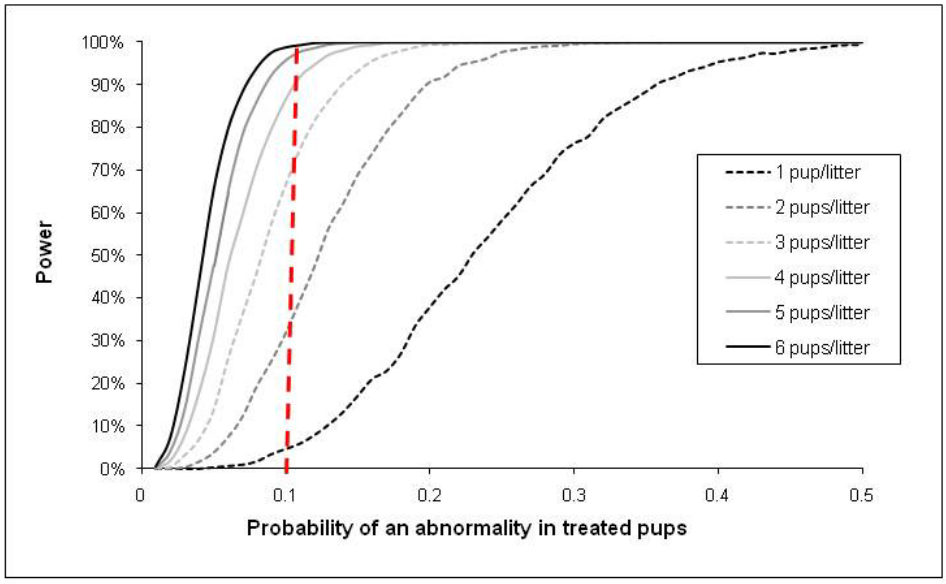
Kinetics of Rat Spermatogenesis



Courtesy of Dr RE Chapin

Power curves for detection of offspring abnormalities with low background incidence (20 litters, 0% background)

Blystone etal (2010) Toxicol Sci **116**(2): 640-646.



10% incidence detected 4.7% of time with 1 pup; 66.4% 3pups; 86.5% 4 pups

Recent NTP Study Design Developments

- Following several NTP workshops, the Program changed its default exposure paradigm to include exposure during pregnancy and early life in rat carcinogenicity studies.
- The NTP has conducted "perinatal cancer bioassays" in the past, but their conduct required special justification. The new default is to undertake such a study unless there is a scientific reason not to do so.
- Before undertaking such a study, some preliminary dose-range finding study would normally be required to ascertain dose levels, using perinatal exposure.

Preliminary Design prior to a Carcinogenicity Study

- Ascertain dose levels for continuous exposure that would allow:
  - Dams to successfully carry their offspring to term
  - Deliver their offspring
  - Successfully raise their offspring to weaning
  - Determination of any target organ toxicity in adults that would preclude the successful conduct of a carcinogenicity study (equiv. 90 d study – 10/sex/group)
- Such a study design could easily be adapted to provide toxicity information on a range of other end points and **maximize the use of the animals already produced** –
- The "Modified One-Generation Study"

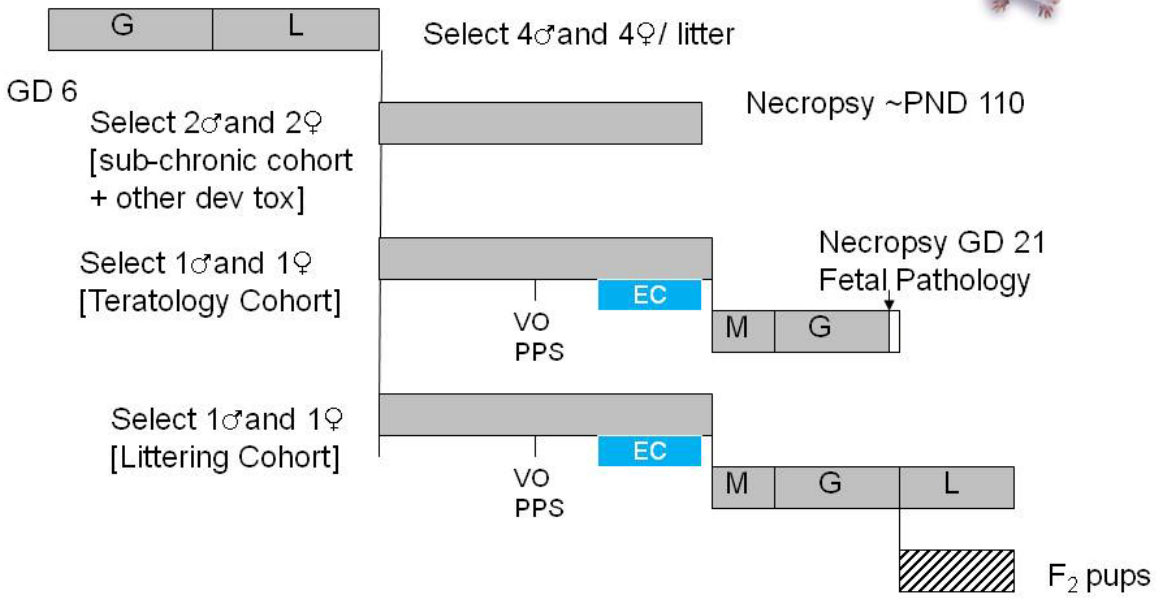
NTP Design

- The basic design has continuous exposure from implantation through to sexual maturity and examines the majority of animals produced.
- It has various interchangeable "cassettes" incorporated from other standard regulatory studies, based on the NTP nomination.
- Typically, the first cohort of offspring is designed to provide information on target organ toxicity, but also has the ability and capacity to evaluate other end points (e.g., immunological or behavioral end points).
- The second cohort of animals evaluates the potential for prenatal developmental toxicity (teratology).
- The third cohort may be used to evaluate breeding and littering for potential examination of the subsequent generation.
- Importantly, **all** F_1 animals after PND 4 are taken to adulthood for pathology examination and there are two of these cohorts that will examine functional effects on reproduction (fertility/ fecundity).

NTP
National Toxicology Program

NTP Modified One-generation Study

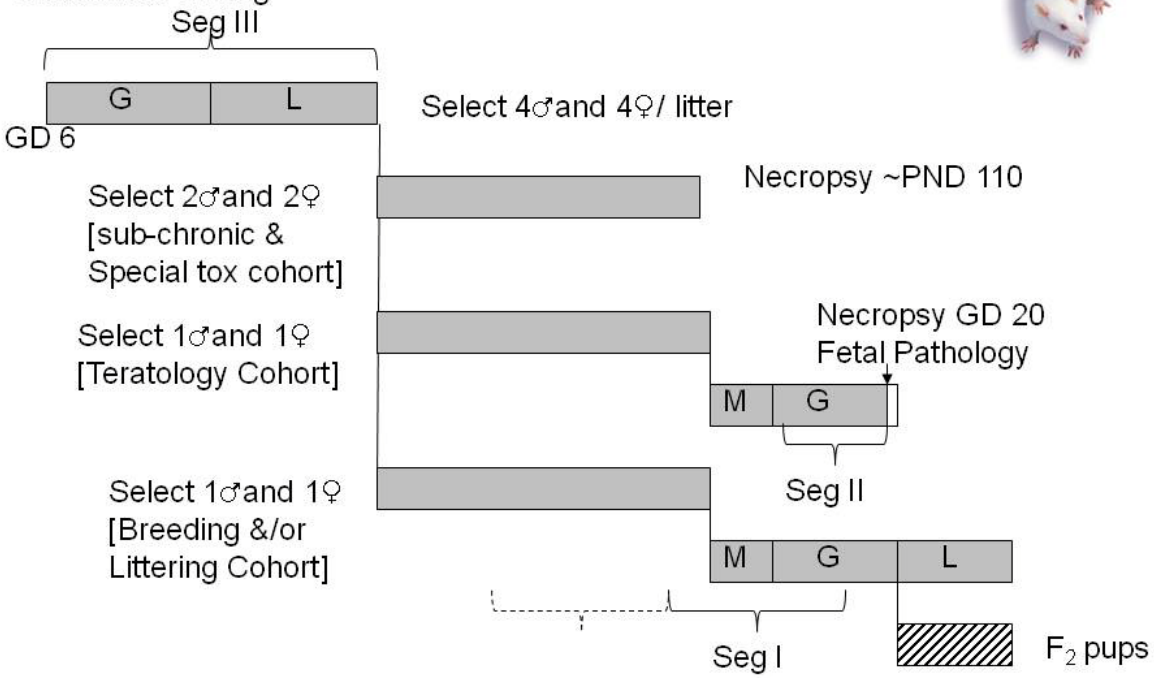
Timed – mated Female Rats min. of 20 litters/gp; 3 dose grps + control



NTP
National Toxicology Program

NTP Modified One-generation Study

Timed – Pregnant Female Rats - 3 dose groups + control
Continuous dosing



How would the MOG be used? The example of Tetrachloroazobenzene (TCAB) rat studies

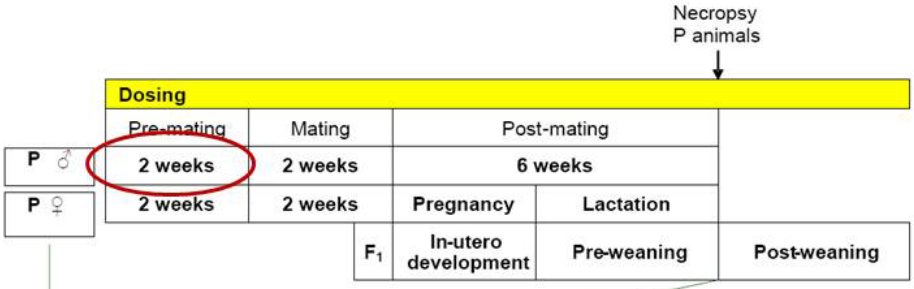
- 14d toxicity
- 90d toxicity
- 2 yr toxicity/ cancer
- Multigeneration reproduction
- Prenatal developmental toxicity
- Combined developmental neurotoxicity and immunotoxicity
- Perinatal pilot
- MOG
- 2 yr toxicity/ cancer (perinatal)

NB need for ADME/ TK and other investigative studies

Other International Developments in Reproduction Study Design

- OECD is developing a new protocol (extended one-generation reproduction study) based on an earlier study design developed by a HESI panel to evaluate agrochemicals.
- Protocol to be used for **all** chemicals as a replacement for the multigeneration reproduction study.
- One of the major drivers is to address testing requirements of REACH (Registration, Evaluation, Authorization and restriction of CHemical substances)
  - 60% of animals needed in a chemical toxicology portfolio will be used for reproduction and developmental toxicity studies.
- The study design employs the use of internal triggers for the measurement of specific end points, or groups of animals.

OECD Proposed Design



Parental generation	Cohort	Designation	Animals/Cohort	Sexual Maturation	Approximate age at necropsy (weeks)
Target is 20 litters per group	1A	Reproductive	20 M +20 F	Yes	13
	1B	Reproductive	20 M +20 F	Yes	14 or 20 if triggered
	2A	Neurotoxicity	10 M +10 F@	Yes	9
	2B	Neurotoxicity	10 M +10 F@	No	3
	3	Immunotoxicity	10 M +10 F@	Yes	8
	Surplus	Spares		No	3

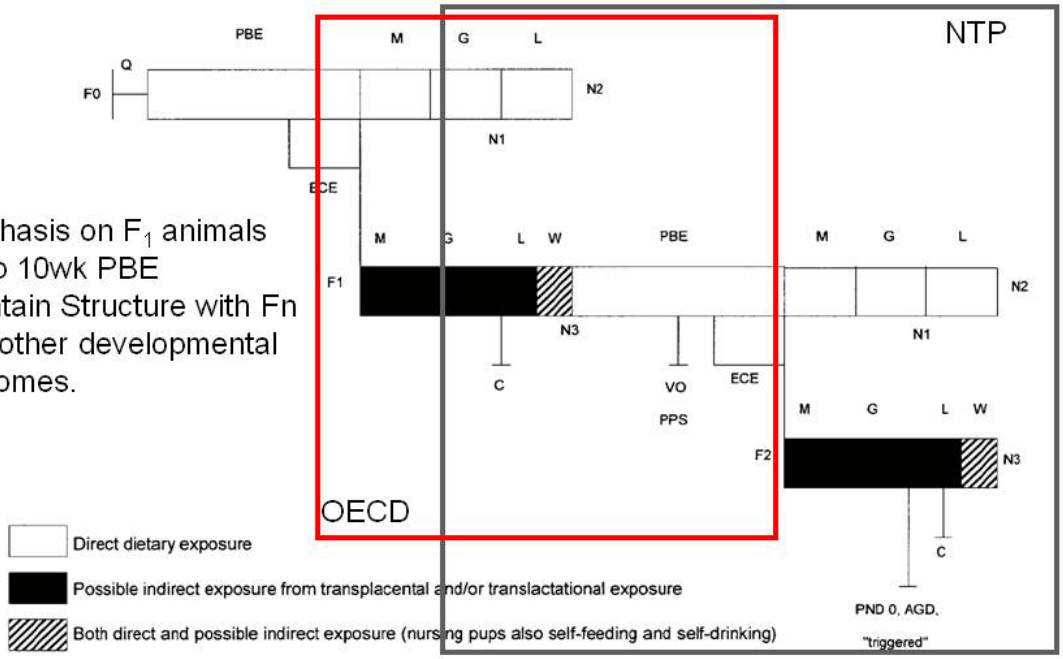
@ one per litter and representative of 20 litters in total where possible

Some of the Triggers for F_1 Breeding on the OECD Study

- P1 (F_0) fertility (# implantations, pregnancy rate, gestational interval) – In the **absence** of a **corresponding**, treatment related reproductive organ **histopathology**.
- F_1 developmental landmarks (AGD, nipple retention, puberty onset, PPS, VO) – Dose-related effects in the **absence of body weight-mediated changes** in these parameters.
  - 4-day advance VO at highest dose level. Will the body weight be decreased in the treated group? **Is this due to toxicity or because they were younger?**
- **When will the data be available to make these decisions?**
  - Study stagger, data collection, statistics, discussions with sponsor and/or RA ?

EPA/ OECD Multigeneration Study

Emphasis on F_1 animals
Keep 10wk PBE
Maintain Structure with Fn
Add other developmental outcomes.



OECD Design Conclusions

- The OECD study is not an improvement over the current multigeneration study.
  - It has many scientific design limitations – some highlighted today
  - **We could have a reproduction study that does not evaluate reproductive function adequately.**
- Failure to breed the F_1 animals routinely is not a comprehensive evaluation of reproduction, nor making the best use of this unique cohort of animals (womb to tomb exposure).
  - **The triggers do not take into consideration the timing of the available information to make good scientific decisions, protective of public health.**

Advantages of the NTP MOG over the OECD Design

- Flexible design
  - Interchangeable cassettes depending on data requirements
- Robust datasets
  - Representative males and females from at least 20 litters per dose group
  - Improved statistical power from evaluating multiple offspring from each litter
- Keep the pre-breed exposure at 10 weeks
  - Based on robust biology, maintains relationship of changes in reproductive structure to function in same animals
- NO "internal" triggers
- Pre-natal developmental toxicity information
- Sub-chronic toxicity information (+ clin path)

Conclusions

- The proposed NTP study design is a robust evaluation of reproductive and developmental toxicity.
  - maximizes the utility of the animals already produced & available for study
  - reduces the overall number of animals employed compared to a multigeneration, or combination of "stand-alone" toxicity studies
- The number of animals employed is comparable to the recently proposed OECD design, but provides much more information on developmental outcomes.
- The design will facilitate NTP's requirement for information on sub-chronic toxicity and dose setting before embarking on a rat perinatal carcinogenesis study.
- We will be able to:
  - Refine our toxicity study designs
  - Replace certain other standard toxicity studies by folding them into this proposed protocol.
  - Reduce overall animal use